JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Reversible Interconversion of CO_2 and Formate by a Molybdenum-Containing Formate Dehydrogenase

Arnau Bassegoda,[†,§] Christopher Madden,[‡,§] David W. Wakerley,[‡] Erwin Reisner,*[,‡] and Judy Hirst*[,†]

[†]Medical Research Council Mitochondrial Biology Unit, Hills Road, Cambridge, CB2 0XY, United Kingdom
[‡]Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom

Supporting Information

ABSTRACT: CO_2 and formate are rapidly, selectively, and efficiently interconverted by tungsten-containing formate dehydrogenases that surpass current synthetic catalysts. However, their mechanism of catalysis is unknown, and no tractable system is available for study. Here, we describe the catalytic properties of the molybdenum-containing formate dehydrogenase H from the model organism *Escherichia coli* (*Ec*FDH-H). We use protein film voltammetry to demonstrate that *Ec*FDH-H is a highly active, reversible electrocatalyst. In each voltammogram a single point of zero net current denotes the CO_2 reduction potential that varies with pH according to the Nernst equation. By quantifying formate production we show that electrocatalytic CO_2 reduction is specific. Our results reveal the capabilities of a Mo-containing catalyst for reversible CO_2 reduction and establish *Ec*FDH-H as an attractive model system for mechanistic investigations and a template for the development of synthetic catalysts.

The efficient reduction of carbon dioxide (CO_2) to generate reduced carbon compounds for use as fuels and chemical feedstocks is an essential requirement for a carbon-based sustainable energy economy.[1] The electrochemical reduction of CO_2, powered by carbon-neutral electricity, would produce liquid fuels that are easier to store and transport than hydrogen, but only limited progress has been made in developing synthetic catalysts to overcome the kinetic and thermodynamic challenges of CO_2 activation. Catalysts developed so far are inefficient and expensive, due to their requirement for high overpotentials or their reliance on noble metals.[2−9]

The rapid, reversible, and specific electrochemical reduction of CO_2 to formate by a tungsten-containing formate dehydrogenase from the anaerobic bacterium *Syntrophobacter fumaroxidans* (*Sf*FDH1) provided the paradigm case for a formate/CO_2 catalyst.[10] *Sf*FDH1 catalyzes the rapid interconversion of CO_2 and formate at the reduction potential for the reaction, establishing it as a thermodynamically reversible catalyst.[11] Therefore, the catalytic mechanism of CO_2 reduction by the W-center in *Sf*FDH1 is a valuable source of information to aid the design of improved synthetic catalysts. However, *Sf*FDH1 itself is intractable for mechanistic studies: cell cultures of *S. fumaroxidans* take several months to achieve low cell densities that provide only minuscule amounts of enzyme; no genetic manipulation is possible; and the enzyme contains an extensive cohort of iron−sulfur (FeS) centers to transfer electrons to and from the active site, making overexpression strategies untenable.[10,12,13] Therefore, a more versatile and robust experimental system is required.

*Sf*FDH1 is a member of the large class of prokaryotic formate dehydrogenases that contain either Mo- or W-cofactors; they also contain a second, independent active site where quinone, protons, or $NAD(P)^+$ react,[14,15] which may be replaced functionally by an electrode to produce an electrocatalyst for CO_2/formate interconversion. The W-containing active site is known to be thermodynamically reversible[10] but is found exclusively in anaerobic bacteria such as *Desulfovibrio*[16] and *Eubacterium*[17] species. Conversely, the thermodynamic reversibility of the more common molybdenum-containing active site remains to be established. Several indications that CO_2 reduction is possible have been reported: a multisubunit Mo-containing FDH from *Desulfovibrio vulgaris* Hildenborough has been reported to reduce CO_2 slowly in solution,[18] and production of formate from CO_2 and H_2 was observed in early whole-cell experiments with *Escherichia coli*[19] (suggesting the formate hydrogenlyase can operate in reverse), and from a putative Mo-containing FDH in the multisubunit H_2-dependent CO_2 reductase of *Acetobacterium woodii*.[20] Here, we define the catalytic properties of the structurally defined Mo-containing formate dehydrogenase H, a component of the formate hydrogenlyase complex in the model organism *E. coli* (*Ec*FDH-H).[21]

*Ec*FDH-H contains a Mo coordinated by a selenocysteine (SeCys) residue and two molybdopterin guanine dinucleotides (MGDs), and just one [4Fe-4S] cluster for electron transfer to and from the active site (Figure 1).[21] It was produced by overexpression in *E. coli* under anaerobic growth conditions[22,23] and purified by adapting a previously reported method[24] (see Supporting Information (SI) for details). All purification and experimental procedures were performed under strictly anaerobic conditions.

Figure 2 shows protein film voltammograms recorded at different pH values with *Ec*FDH-H adsorbed on the surface of a graphite-epoxy rotating disk working electrode (geometric surface area 0.07 cm^2; see SI for details). *Ec*FDH-H catalyzes the reversible interconversion of CO_2 and formate with electrocatalytic characteristics similar to those observed previously for *Sf*FDH1.[10] Each set of voltammograms slices

Received: August 22, 2014
Published: October 17, 2014

   dx.doi.org/10.1021/ja508647u | J. Am. Chem. Soc. 2014, 136, 15473−15476

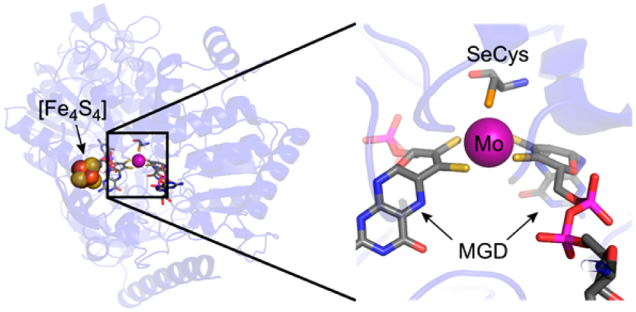

Figure 1. Structure of *Ec*FDH-H showing the active site consisting of Mo coordinated by a SeCys and two MGDs and the [4Fe-4S] cluster that transports electrons to and from the active site. Figure generated with PyMOL from 1AA6 pdb.[21]

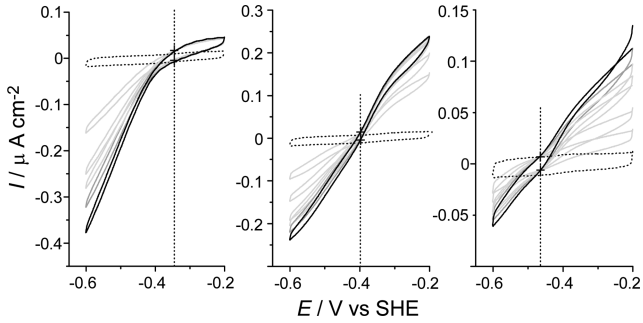

Figure 2. Cyclic voltammograms showing reversible CO_2 reduction and formate oxidation by *Ec*FDH-H adsorbed on a graphite-epoxy electrode, pH 6.0 (left), 6.8 (middle), and 8.0 (right). The points of intersection (marked with crosses) define the reduction potentials for the CO_2/formate interconversion (vertical lines). First voltammetric cycles are shown in black and subsequent cycles (2−4, 10, and 20) in gray. Voltammograms recorded in the absence of substrates are shown as dashed traces. Conditions: 10 mM CO_2,[25] 10 mM formate, 25 mM of each of four pH buffers (acetate, MES, HEPES, and TAPS), voltammetric scan rate 25 mV s^{-1}, electrode rotation rate 2000 rpm, 23 °C.

cleanly through unique zero-current points, traced out as points of intersection as the current decays during successive scans. The zero-current points denote the thermodynamic reduction potential for the CO_2/formate interconversion (net formate oxidation occurs at more positive potentials and net CO_2 reduction at more negative potentials), demonstrating that the electrocatalytic reaction is thermodynamically reversible.[11] Both the oxidation and reduction currents increase rapidly as the overpotential is increased but do not reach potential-independent limiting currents within the accessible potential range. This behavior is typical of highly catalytically active enzymes, for which the electrocatalytic rate is limited by interfacial electron transfer.[11]

Figure 3 shows how the measured CO_2 reduction potentials vary with pH, and that they are consistent with potentials calculated using the Nernst equation and known p*K* values.[10,26] They also match values measured previously using *Sf*FDH1,[10] with a small decrease in the predicted value of $E^{0\prime}$ attributable to the lower temperature used here. The data in Figure 3 are key evidence in establishing Mo-containing *Ec*FDH-H as a catalyst specific for CO_2/formate interconversion as well as a demonstration that catalysis remains reversible over a wide range of conditions.

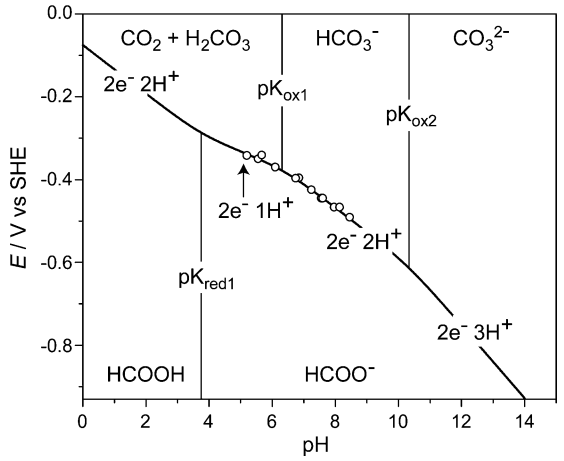

Figure 3. Reduction potentials for the CO_2/formate interconversion measured using *Ec*FDH-H, superimposed on a Pourbaix diagram to show the most stable species present under each condition. The reduction potentials (measured as illustrated in Figure 2) were recorded in 10 mM formate and 10 mM CO_2[25] at 23 °C and fitted to the Nernst equation (solid line)[10] using pK_{red1} = 3.75, pK_{ox1} = 6.39, pK_{ox2} = 10.32,[10,26] and $E^{0\prime}$ = −0.075 V.

To confirm the selectivity of catalysis, bulk CO_2 reduction was carried out using a graphite-epoxy "pot" working electrode (geometric surface area ∼5.3 cm^2) with *Ec*FDH-H adsorbed to the surface. The current was recorded during electrolysis periods of 1−2 h at −0.5 and −0.6 V vs SHE (∼110 mV and 210 mV overpotential, respectively), and the total charge passed calculated by integration of the current over time (see Figure S2). Following electrolysis, analysis by ion chromatography revealed formate as the single observable product, formed with a Faradaic efficiency of 101.7 ± 2.0% (standard error measurement, n = 3). These data confirm formate as the quantitative product of the electrocatalytic reduction of CO_2 to formate by *Ec*FDH-H.

Despite the Mo-containing *Ec*FDH-H and the W-containing *Sf*FDH1 being similarly capable of the electrocatalytic interconversion of CO_2 and formate, their abilities to catalyze in solution-based assays are strikingly different. Standard FDH solution assays utilize either benzyl viologen (BV^{2+}) or methyl viologen (MV^{2+}) as the redox partner and monitor either formation (reduction of MV^{2+}) or consumption (oxidation of MV^{+}) of the blue radical-cation reduced viologen (MV^{+}), coupled to formate oxidation or CO_2 reduction, respectively.[12,27] Table 1 compares solution assay and electrochemical data from *Sf*FDH1 and *Ec*FDH-H.

Both *Ec*FDH-H and *Sf*FDH1 catalyze the rapid oxidation of formate coupled to the reduction of BV^{2+}, an electron acceptor with a reduction potential more positive than that of CO_2 (−0.36 V vs SHE).[28] For this reason, BV^{+} is unsuitable for CO_2 reduction assays. Only *Sf*FDH1 is capable of rapid formate oxidation coupled to the reduction of MV^{2+}, an electron acceptor with a lower reduction potential (−0.45 V vs SHE)[28] that is comparable to that of CO_2. Furthermore, solution measurements of CO_2 reduction using MV^{+} revealed rapid formate production only by *Sf*FDH1. Assays with *Ec*FDH-H yielded turnover numbers for CO_2 reduction below 1 s^{-1}, more than 2 orders of magnitude slower than the rate of BV^{2+}-linked formate oxidation, or of the rates of both reactions catalyzed by *Sf*FDH1. This result is likely the reason why CO_2 reduction by this Mo-containing FDH has not been observed previ-

dx.doi.org/10.1021/ja508647u | J. Am. Chem. Soc. 2014, 136, 15473−15476

Table 1. Comparison of Catalysis by *Sf*FDH1 and *Ec*FDH-H in Solution Assays and Electrochemically (pH 7.5 ± 0.1, 23 °C)

reaction	W-*Sf*FDH1	Mo-*Ec*FDH-H
formate + MV^{2+}	1500 s^{-1}[a]	4 s^{-1}
formate + BV^{2+}	est. 1100 s^{-1}[b]	160 s^{-1}
CO_2 + MV^{+}	500 s^{-1}[a]	<1 s^{-1}
formate + electrode[c]	160 $\mu A\ cm^{-2}$[a]	180 $\mu A\ cm^{-2}$
CO_2 + electrode[c]	5 $\mu A\ cm^{-2}$[a]	80 $\mu A\ cm^{-2}$

[a]Taken from ref 10. [b]Value estimated from published values,[12] supported by preliminary in-house data. [c]250 mV overpotential for *Sf*FDH1, 150 mV for *Ec*FDH-H. Solution assays contained 1 mM BV^{2+} or MV^{2+} for formate oxidation or 0.1 mM MV^{+} for CO_2 reduction. All experiments used 10 mM formate or 10 mM CO_2.[25]

ously,[24,27,29] leading to the general concept that the Mo-containing active site does not catalyze in a thermodynamically reversible manner.

Electrochemically, both enzymes catalyze in both directions with significant rates. In both cases formate oxidation increases, relative to CO_2 reduction, as the pH is increased, but at pH 7.5 *Ec*FDH-H is biased more strongly toward CO_2 reduction than *Sf*FDH1 (Table 1), suggesting that the "operating potential"[11] of *Ec*FDH-H is more negative than that of *Sf*FDH1. Although this interesting suggestion is contrary to expectations that the W-center should operate at a lower potential than the Mo-center, intramolecular electron transfer to and from the active site may also influence the catalytic bias.[11]

The reason why *Ec*FDH-H, despite being such a good electrochemical catalyst, is unable to catalyze CO_2 reduction by MV^{+} or formate oxidation by MV^{2+} is intriguing. We suggest two possibilities. First, it may be purified in an inactive state that cannot be recovered easily in solution-based assays. Attempts to use different conditions and pretreatments to reactivate the enzyme have failed to substantiate this suggestion, but catalytic lag phases observed in formate oxidation assays by *Sf*FDH1 (which can be avoided by pretreatment with MV^{+}) suggest that inactive states of FDH enzymes can be formed. Second, the activity of *Ec*FDH-H may be dominated by the single [4Fe-4S] cluster that transfers electrons to and from the active site. During formate oxidation the Mo-center readily reduces the cluster, but the cluster (having, we expect, a more positive reduction potential) is able to pass its electron efficiently only to BV^{2+}, not MV^{2+}. Similarly, for CO_2 reduction, MV^{+} readily reduces the cluster, but the electron tends to remain on the higher-potential cluster (blocking further electron transfers from MV^{+}), rather than move to the active site. In contrast, on the electrode surface the abundance of electrons with sufficient driving force overcomes the FeS barrier to CO_2 reduction by backfilling the oxidized cluster immediately, when the electron moves otherwise transiently to the active site (and similarly, for formate oxidation it takes the electron from the cluster at the active site potential). In contrast to *Ec*FDH-H, *Sf*FDH1 contains around 10 FeS clusters[10] to buffer electron supply and demand. Our results highlight the problems of relying on inefficient and slow redox mediators to report on catalysis by a rapidly catalyzing, buried active site.

The reduction of CO_2 to liquid fuel products is currently of much interest, and there is a strong requirement for catalysts that operate efficiently, selectively and under mild conditions. Some ruthenium, iron, manganese, and copper based catalysts are able to reduce CO_2 electrochemically, but large overpotentials are typically required and their efficiency is low.[2−9] In contrast, formate dehydrogenases catalyze the two electron reduction of CO_2 directly to energy-rich formic acid with high selectivity, under mild conditions, with little overpotential requirement, and elucidation of their catalytic mechanism may inform the development of improved synthetic catalysts. Tungsten-containing *Sf*FDH1 from *S. fumaroxidans* set an important paradigm but is intractable for in-depth studies. Here we have demonstrated that molybdenum-containing *Ec*FDH-H from *E. coli* is also capable of reversible, specific, and efficient CO_2 reduction, so the Mo-center is capable of reversible CO_2 reduction and provides a new blueprint for synthetic catalyst design. Based on its simplicity and relative ease of production and manipulation, we establish *Ec*FDH-H as a new model system of choice for mechanistic investigations of enzymatic CO_2 reduction.

■ ASSOCIATED CONTENT

Supporting Information

Experimental methods for protein preparation, solution assays, and electrocatalysis experiments, and comparison with the kinetic data of Axley and Grahame.[27] This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

jh@mrc-mbu.cam.ac.uk
reisner@ch.cam.ac.uk

Author Contributions

[§]These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Biotechnology and Biological Sciences Research Council (grant nos. BB/I026367/1 to J.H. and BB/J000124/1 to E.R.), the Medical Research Council (grant no. U105663141 to J.H.), and the Engineering and Physical Sciences Research Council (grant number EP/H00338X/2 to E.R.). We thank Professor J. H. Golbeck from Pennsylvania State University for providing *E. coli* strain JG0205 and an initial expression plasmid for *Ec*FDH-H.

■ REFERENCES

(1) Appel, A. M.; Bercaw, J. E.; Bocarsly, A. B.; Dobbek, H.; DuBois, D. L.; Dupuis, M.; Ferry, J. G.; Fujita, E.; Hille, R.; Kenis, P. J. A.; Kerfeld, C. A.; Morris, R. H.; Peden, C. H. F.; Portis, A. R.; Ragsdale, S. W.; Rauchfuss, T. B.; Reek, J. N. H.; Seefeldt, L. C.; Thauer, R. K.; Waldrop, G. L. *Chem. Rev.* **2013**, *113*, 6621−6658.

(2) Kang, P.; Cheng, C.; Chen, Z.; Schauer, C. K.; Meyer, T. J.; Brookhart, M. *J. Am. Chem. Soc.* **2012**, *134*, 5500−5503.

(3) Huff, C. A.; Sanford, M. S. *ACS Catal.* **2013**, *3*, 2412−2416.

(4) Hull, J. F.; Himeda, Y.; Wang, W.-H.; Hashiguchi, B.; Periana, R.; Szalda, D. J.; Muckerman, J. T.; Fujita, E. *Nat. Chem.* **2012**, *4*, 383−388.

(5) Kang, P.; Meyer, T. J.; Brookhart, M. *Chem. Sci.* **2013**, *4*, 3497−3502.

(6) Costentin, C.; Drouet, S.; Robert, M.; Savéant, J.-M. *Science* **2012**, *338*, 90−94.

(7) Sampson, M. D.; Nguyen, A. D.; Grice, K. A.; Moore, C. E.; Rheingold, A. L.; Kubiak, C. P. *J. Am. Chem. Soc.* **2014**, *136*, 5460−5471.

(8) Smieja, J. M.; Sampson, M. D.; Grice, K. A.; Benson, E. E.; Froehlich, J. D.; Kubiak, C. P. *Inorg. Chem.* **2013**, *52*, 2484−2491.
(9) Haines, R. J.; Wittrig, R. E.; Kubiak, C. P. *Inorg. Chem.* **1994**, *33*, 4723−4728.
(10) Reda, T.; Plugge, C. M.; Abram, N. J.; Hirst, J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 10654−10658.
(11) Armstrong, F. A.; Hirst, J. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 14049−14054.
(12) de Bok, F. A. M.; Hagedoorn, P.; Silva, P. J.; Hagen, W. R.; Schiltz, E.; Fritsche, K.; Stams, A. J. M. *Eur. J. Biochem.* **2003**, *270*, 2476−2485.
(13) de Bok, F. A. M.; Luijten, M. L. G. C.; Stams, A. J. M. *Appl. Environ. Microbiol.* **2002**, *68*, 4247−4252.
(14) Hille, R.; Hall, J.; Basu, P. *Chem. Rev.* **2014**, *114*, 3963−4038.
(15) Johnson, M. K.; Rees, D. C.; Adams, M. W. W. *Chem. Rev.* **1996**, *96*, 2817−2840.
(16) Raaijmakers, H.; Macieira, S.; Dias, J. M.; Teixeira, S.; Bursakov, S.; Huber, R.; Moura, J. J. G.; Moura, I.; Romão, M. J. *Structure* **2002**, *10*, 1261−1272.
(17) Graentzdoerffer, A.; Rauh, D.; Pich, A.; Andreesen, J. R. *Arch. Microbiol.* **2003**, *179*, 116−130.
(18) da Silva, S. M.; Pimentel, C.; Valente, F. M. A.; Rodrigues-Pousada, C.; Pereira, I. A. C. *J. Bacteriol.* **2011**, *193*, 2909−2916.
(19) Woods, D. D. *Biochem. J.* **1936**, *30*, 515−527.
(20) Schuchmann, K.; Müller, V. *Science* **2013**, *342*, 1382−1385.
(21) Boyington, J. C.; Gladyshev, V. N.; Khangulov, S. V.; Stadtman, T. C.; Sun, P. D. *Science* **1997**, *275*, 1305−1308.
(22) Zhang, J. W.; Butland, G.; Greenblatt, J. F.; Emili, A.; Zamble, D. B. *J. Biol. Chem.* **2005**, *280*, 4360−4366.
(23) Hopper, S.; Babst, M.; Schlensong, V.; Fischer, H.-M.; Hennecke, H.; Böck, A. *J. Biol. Chem.* **1994**, *269*, 19597−19604.
(24) Axley, M. J.; Grahame, D. A.; Stadtman, T. C. *J. Biol. Chem.* **1990**, *265*, 18213−18218.
(25) Note that we refer to 'CO_2 reduction' but (due to the very nonideal behavior of CO_2 as a solute) have defined the total concentrations of CO_2 and carbonate species present by addition of sodium carbonate.
(26) Palmer, D. A.; Van Eldik, R. *Chem. Rev.* **1983**, *83*, 651−731.
(27) Axley, M. J.; Grahame, D. A. *J. Biol. Chem.* **1991**, *266*, 13731−13736.
(28) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1755.
(29) Enoch, H. G.; Lester, R. L. *J. Biol. Chem.* **1975**, *250*, 6693−6705.